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19 ABSTRACT (Continue on reverse if necessary and identify by block number) The interactions between the marine amoeba <u>Trichosphaerium</u> Am-I-7 and a variety of symbiotic and non-symbiotic dinoflagellates is being studied. The amoeba may eat specific available algae, ignore some species, and phagocytose other species but not digest them. Non symbiotic dinoflagellates were quickly digested. Symbiotic dinoflagellates especially of the genus <u>Symbiodinium</u> , were either avoided or phagocytosed to different extents but were not digested. Of the symbiotic dinoflagellates <u>Symbiodinium</u> species #8 was especially interesting. The amoebae packed themselves with the alga and maintained live algae in perialgal vacuoles. The amoeba is selective both in its uptake of particles and in its ability to retain specific algae undigested. About 10% of the photosynthetic metabolites of the algae were translocated, and incorporated in amoebal cytoplasm. Two types of amoebal vacuoles were identified by differential binding of fluorescent lectins. Digestive food vacuoles bound differentially to RCA120. N. ...			
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TITLE: AN AMOEBA/ZOOXANTHELAE CONSORTIUM AS A MODEL SYSTEM FOR ANIMAL/ALGAL INTERACTION.

PRINCIPLE INVESTIGATOR: Dr. Miriam Polne-Fuller

CO-PRINCIPLE INVESTIGATORS: Drs. Aharon Gibor and Robert Trench

CONTRACTOR: Marine Biotechnology Center, Biological Sciences,  
University of California Santa Barbara, CA 93106.

START DATE: 1 July 1988

RESEARCH OBJECTIVES:

The major goals of this project are:

1. To investigate the interaction between Trichosphaerium Am-I-7 and symbiotic dinoflagellates, specifically Symbiodinium species #8 with which the amoebae created an unusual consortium.
2. To study the mechanism(s) by which the amoebae recognize the different algal species.
3. To investigate the mechanism(s) by which the algae "resist" being digested by the amoeba.

We have now confirmed that the interaction between the host amoeba and the intracytoplasmic dinoflagellates is beneficial to both partners.



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## FIRST YEAR STUDIES:

Our studies at this stage centered on defining the interactions between the host amoeba and the algal cells which are internalized in it.

The relationship established between the animal (the amoebae) and the algae, i.e. the consortium, is beneficial to both partners and prevents death from starvation.

This statement is based on observations in mixed cultures of amoebae and the alga Symbiodinium #8. Mixed cultures of the two organisms were maintained without changing of the medium for over two years. Live and active amoebae with internalized algal cells survived well in such cultures. Under identical culture conditions, separately cultured, starved amoebae and algae die within 1-2 months. Thus the interaction is certainly contributing to the survival of both species.

## Translocation of metabolites:

Carbon compounds from the internalized algal cells were transferred to the host cells. This was demonstrated by a series of experiments exposing the consortium as a unit and each of its partners, separately, to radioactive bicarbonate in the light. The algal cells fixed carbon photosynthetically while the amoebae did not take label in any form. About 10% of the algal fixed carbon, (estimated from scintillation counts, and radiographs) were transferred into the amoeba cytoplasm within 2 hours.

The appearance of labeled metabolites in the amoebae during this short period indicates secretion of metabolites from the algae into the amoeba rather than digestion of some of the possibly broken internalized algal cells. Such digestion when occurred, was a much slower process as indicated from microscopic observations.

## Recognition and uptake of Algae:

Uptake rates of 4 species of Symbiodinium by the amoeba were investigated. The algae were presented to the amoebae in their culture vessels, and the number of algae phagocytosed by the amoebae were recorded daily. Already at this initial stage of interaction there were clear differences between the uptake rates of the different algae. Symbiodinium species #8 was the leading algae with the largest number of algal cells taken daily per amoeba (Fig. 1). The other Symbiodinium species were taken up at a slower rate (Fig. 1). The preference of Symbiodinium species #8 was also evident in the final result of phagocytosis when the amoebae filled themselves with algae (Fig. 2), a phenomenon which did not occur with any of the other species of dinoflagellates tested.

To understand the relationships established between digestible and nondigestible algal cells which are incorporated into the animal cells we undertook to study the physiology of the internalized vacuoles. We are searching for biochemical markers by which to distinguish between a "digesting" vacuole and a "nondigesting compartment". Such a marker will be essential for in vitro isolation of these specific cellular compartments.

#### Lectin affinity:

A variety of fluorescent lectins were tested as markers in the amoebae cells. The different lectins did show specificities and bound to different components of the amoebae. Con-A bound to external cell membranes of the amoebae. Nuclei and nucleoli and centrosomes were stained by a variety of other lectins (submitted for publication).

The most useful lectin for this project was ricin communis agglutinin (RCA120) which bound to digestive food vacuoles of the amoeba and not to other vacuoles or other cell membranes. This differential staining will serve as a marker for digestive activities versus resistance to digestion. Using the lectin marker we will now attempt at following the fate of membrane recycling in the amoeba. It will be especially useful in future studies of transformation and selection of resistant strains from digested species, and vice versa.

#### Vital Dyes staining:

To determine the viability of the internalized algal cells we used vital dyes, especially useful was Neutral red staining. Intact algal cells excluded Neutral red while damaged cells stained dark red. In the amoeba's cytoplasm, most of the algae which looked intact and were viable upon removal from the amoebae did not stain with NR. About 0.5-2% of the algae inside the amoebae did stain with NR, and the vacuoles containing these algae also stained with RCA120, both suggesting that the cells were being digested.

This unique interaction between an amoeba and symbiotic dinoflagellates, especially that with Symbiodinium #8 had allowed combined cultures of the two species to maintain themselves for over two years with no changes or additions to the medium.

## Microscopic Studies of Consortia:

On the level of the electron microscope the algae in the amoebal cytoplasm looked intact. The algae were always packed in membrane bound vacuoles, usually one alga per vacuole. Some vacuoles did contain two algal cells in stages of cell division. Whether the dividing algal cells were taken up as such or initiated division in the amoebae is not clear as yet.

## Light Microscopy:

Nomarsky microscopy indicated that although most algae were packed individually, occasional vacuole contained up to 5 algal cells. It will be interesting to follow such a multiple-algal-vacuole over time, to determine whether the algal cells become separated into, or collected from individual compartments.

## WORK PLAN FOR SECOND YEAR:

We are continuing experiments in search for the cues involved in the uptake and maintenance of algae in the amoebal cytoplasm.

## Cytological studies:

1. Biochemical treatments for modifications of the cell surface of live algal cell before presentation to the amoeba will be undertaken. By enzyme treatments, and by masking cell surfaces with various inert substances, we will remove, and obscure, components of the outer surface, and evaluate changes in the rate of uptake of and maintenance of the algae by the host amoeba. Preliminary experiments of masking algal surfaces with various lectins indicated that although the initial uptake of S. #8 by the amoebae was not altered, all 6 lectins which were tested did effect the retention and maintenance of the algae.

2. We will test the interaction between these two organisms as the physiology of the algae is changed. Possible changes in uptake rates and algal retention will be monitored during inhibition of specific stages of the photosynthetic pathway, effecting production of sugars. Possible changes in uptake rates and algal retention will be also monitored at various light levels: below, at, and above the photosynthetic saturation level.

3. The ability of the algal-packed-amoebae to sense light and possibly migrate to areas of the culture chamber where photosynthetic light levels are optimal, will also be tested.

#### Microscopy and Time-Lapse:

4. We are in the process of acquiring a time-lapse, image-analysis equipment which will enable us to follow directly, the fate of algal cells within an amoeba. Having time laps and image enhancement capabilities will also enable us to observe, for extended time, the slow action of the phagocytosis of the different species of dinoflagellates. This will allow the observation of the physical process of interaction before the decision to take up or to avoid an algal cell is made by the amoeba.

5. It will also enable us to observe possible changes in uptake behaviour after the different species of algae will be treated to change the properties of their surfaces.

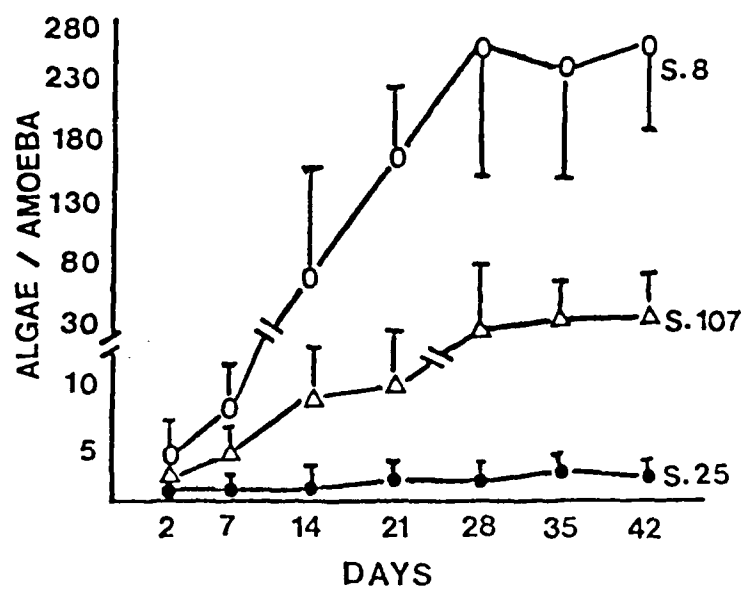


Fig. 1. UPTAKE RATES OF 3 DIFFERENT SPECIES OF SYMBIODINIUM. Symbiodinium#8 was taken up the fastest and in largest numbers. S. #107 was second, and only few cells of S. #25 were taken.

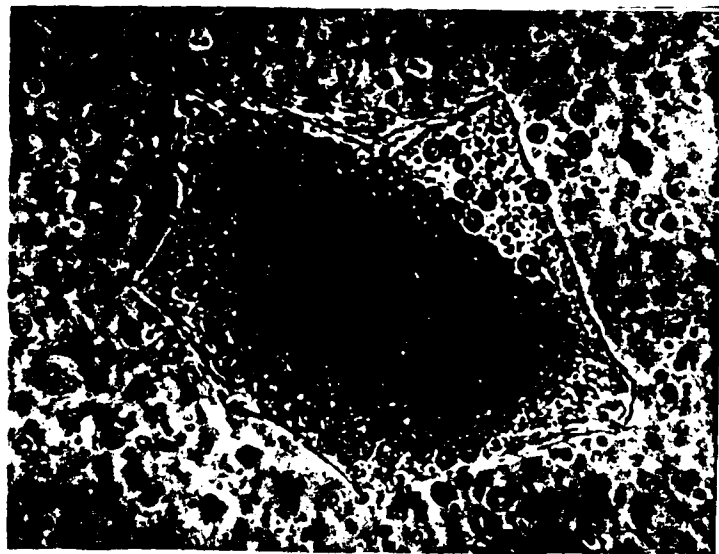


Fig. 2. AMOEBA FULL OF SYMBIODINIUM SPECIES #8.

## PUBLICATIONS

### Abstracts:

Polne-Fuller, M. Nuclear division in the marine amoeba Trichosphaerium Am-I-7. Abstract of the International meeting of the Society of Protozoologists, University of Ill. Champegin/Urbana. 1988

Gibor, A., Polne-Fuller, M., Rogerson, A., and Amano H. Biological potentials of a newly isolated marine amoeba Trichosphaerium Am-I-7. Abstract of the annual meeting of the International Symposium for Marine Biotechnology, summer 1989, Kyoto, Japan.

### Manuscripts in preparation:

M. Polne-Fuller and Aharon Gibor. Selective grazing by the marine amoeba Trichosphaerium Am-I-7. for J.Protozoology.

M. Polne-Fuller, A. Rogerson, R. Trench and A. Gibor. Selective uptake and digestion of four species of the symbiotic dinoflagellate Symbiodinium by the marine amoeba Trichosphaerium Am-I-7. for Endocytobiologia.

A. Rogerson, M. Polne-Fuller, R. Trench, and A. Gibor. A laboratory induced association between the marine amoeba Trichosphaerium Am-I-7 and the dinoflagellate Symbiodinium. for Symbiosis.

A. Rogerson, M. Polne-Fuller, R. Trench, and A. Gibor. Lectines staining as markers of amoebae. for Hydrobiologia.

### TRAINING ACTIVITIES:

One postdoctorate fellow and three undergraduate students are partially supported by this contract.

### AWARDS:

Appointment of Miriam Polne-Fuller as a reviewer on the editorial board for the Journal of Protozoology.

Appointment of Miriam Polne-Fuller as an Adjunct Professor of Biology, at the Department of Biological Sciences, University of California, Santa Barbara.